

Claims

1. A multi-well assay for identifying a compound inhibiting the replication cycle of a micro-organism comprising the subsequent steps of:
  - 5 a) preparing a multi-well comprising micro-organism-coated host cells,
  - b) initiating at time  $t$  micro-organism infection and replication in said micro-organism-coated host cells such that micro-organism infection and replication is initiated synchronically in all host cells,
  - c) bringing at time  $t + \Delta t$  a candidate compound at one or more concentrations into
  - 10 contact with a part of the host cells,
  - d) repeating step c) after a time interval of  $\Delta t$  for another part of said host cells,
  - e) optionally repeating steps c) and d) using one or more other candidate compounds at one or more concentrations, and
  - f) determining whether said candidate compound has inhibited micro-organism
  - 15 replication in said host cells.
2. The assay according to claim 1 wherein said micro-organism is HIV.
3. The assay according to claims 1 and 2, whereby  $\Delta t$  is shorter than the time required
- 20 for passing from one stage to another stage in the micro-organism replication cycle.
4. The assay according to any one of claims 2 to 3, whereby  $\Delta t$  is shorter than the time required for passing from the entry stage to the reverse transcription stage in the micro-organism replication cycle.
- 25 5. The assay according to any one of claims 2 to 4, whereby the compound is identified at inhibition of any one of the HIV entry steps: CD4 receptor attachment phase, co-receptor binding phase, and membrane fusion events.
- 30 6. The assay according to any one of claims 1-4, wherein  $\Delta t$ , at which compounds are repeatedly added to the multi-well, comprises 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 60, 120, 240 or 360 minutes.
7. The assay according any one of claims 1-6, whereby steps c) to e) are performed
- 35 under constant reaction conditions including under a  $\text{CO}_2$ -concentration of 5 %, a relative humidity comprised between 95 and 100% and a temperature of 37°C.

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8. The assay according to any one of claims 1 to 4 and 6 to 7, whereby micro-organism replication in said host cells is initiated at time t by simultaneously bringing all cells at a temperature suitable for initiating micro-organism infection and replication.

5 9. The assay according to any one of claims 1 to 8, whereby said multi-well is prepared by the steps of

- a) coating host cells with a micro-organism at a high multiplicity of infection,
- b) removing unadsorbed micro-organism, and
- c) bringing said micro-organism-coated host cells onto said well.

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10. The assay according to any one of claims 1-9, whereby said host cells in said multi-well are able to express a gene encoding a detectable marker.

11. The assay according to claim 10, wherein a vector that expresses a gene encoding a detectable protein under the control of a HIV responsive promoter is introduced in said host cells.

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12. The assay according to any one of claims 1-11, wherein said micro-organism is labeled with a detectable protein.

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13. The assay according to any one of claims 1-12, whereby determination of said candidate compound is performed by detecting the presence or absence of said detectable marker.

25 14. The assay according to claim 13, wherein the presence or the absence of said detectable marker is detected by means of digital imaging techniques.

15. An apparatus for carrying out an assay according to any one of claims 1-14, comprising:

- 30 - a support suitable for supporting a multi-well comprising micro-organism - coated host cells, optionally moving in one or more directions,
- one or more vials for containing a suspension of micro-organism,
- one or more vials for containing one or more compounds,
- pipetting means for dispensing micro-organisms in said multi-well, optionally moving in one or more directions,
- 35 - pipetting means for dispensing one or more compounds in said multi-well, optionally moving in one or more directions,

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- pipetting controlling means for controlling dispensing by said pipetting means, and
- environment controlling means for keeping conditions in said apparatus constant while bringing one or more compounds into contact with the host cells.

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16. The apparatus according to claim 15 wherein said micro-organism is HIV.
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17. The apparatus according to any one of claims 15 and 16 further comprising a temperature-controlled multi-well support.
18. The apparatus according to any one of claims 15 to 17 further comprising an insulating cover.
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19. The apparatus according to any one of claims 15 to 18 wherein the circuitry of pipetting and environment controlling means are sealed against high humidity.
20. Compounds identifiable with an assay according to any one of claims 1 to 14.
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21. Pharmaceutical composition comprising a therapeutically effective amount of one or more compounds identifiable with an assay according to any one of claims 1 to 14 and a pharmaceutically acceptable excipient.
22. Use of a compound, identifiable with an assay according to any one of claims 1 to 14, as a medicament.
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23. Use of a compound, identifiable with an assay according to any one of claims 1 to 14, for the manufacture of a medicament for treating infectious diseases.
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24. A method of treating AIDS, comprising administering a therapeutically effective amount of a compound identifiable with an assay according to any one of claims 2 to 14 to a patient in need thereof.